

Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

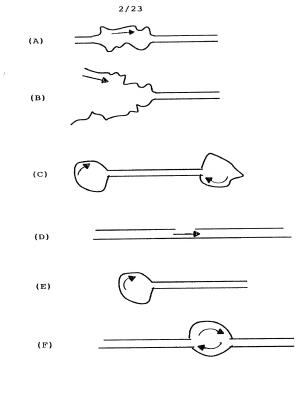


Figure 2 (A-F)

Functional Forms of the Construct

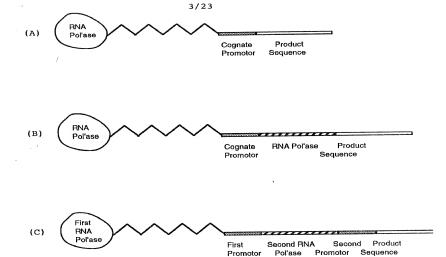


Figure 3 (A-C)

Three Constructs with an RNA Polymerase Covalently Attached to a Transcribing Cassette

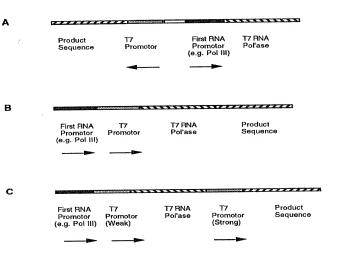


Figure 4 (A-C)

Three Constructs with Promoters for Endogenous RNA Polymerase

1201 CAAAGATGAG

M13mp18. Seq Length: 7250 AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG 1. CTCGCGCCCCC AGGTTATTGA 51. AAATGAAAAT ATAGCTAAAC CCATTTGCGA **AATGTATCTA** 101. ATGGTCAAAC TAAATCTACT **CGTTCGCAGA** ATTGGGAATC **AACTGTTACA** 151. TGGAATGAAA CTTCCAGACA CCGTACTTTA GTTGCATATT TAAAACATGT AGCAATTAAG CTCTAAGCCA 201 TGAGCTACAG CACCAGATTC TOOGCAAAAA 251 TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TOCTGACCTG CTTCCCGCTCT **GGTTCGCTTT** GAAGCTCGAA TTAAAACGCG 301. TTGGAGTTTG 351. **ATATTTGAAG** TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTIT 401. **TGATTTATGG** TTTCTGAACT TTTGAGGGGG 451. **TCATTCTCGT** GTTTAAAGCA **ATTCAATGAA** TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT **AAACATTTTA** 501. 551. CTATTACCCC CTCTGGCAAA ACTICITITIE CAAAAGCCTC TCGCTATTTT 601. **GGTTTTTATC** GICGICIGGT **AAACGAGGGT TATGATAGTG** TTGCTCTTAC TATGCCTCGT AATTCCTTTT **GCCGTTATGT ATCTGCATTA** GTTGAATGTG 701. **GTATTCCTAA ATCTCAACTG** ATGAATCTTT CTACCTGTAA TAATGTTGTT COGTTAGTTC **GTTTTATTAA** CGTAGATTTT TCTTCCCAAC 751. GTOCTGACTG 801. GTATAATGAG CCAGTTCTTA **AAATOGCATA** AGGTAATTCA CAATGATTAA 851. AGTTGAAATT AAACCATCTC AAGCCCAATT TACTACTCGT TCTCGTGTTC 901. TOGTCAGGGC **AAGCTTATT** CACTGAATGA GCAGCTTTGT TACGTTGATT 951. TGGGTAATGA ATATCCGGTT CTTGTCGAAG **ATTACTCTTG ATGAAGGTCA** 1001 GCCAGCCTAT **COCCUTEGIC** TGTACACCGT TCATCTGTCC TCTTTCAAAG 1051 TTGGTCAGTT COGTTCCCTT ATGATTGACC GTCTGCGCCT COTTCCCGCCT 1101 AAGTAACATG GAGCAGGTOG CGGATTTCGA CACAATTTAT CACCOCCATICA 1151 TACAAATCTC OGTTGTACCTT TGTTTCGCGC TTGGTATAAT COCTOCCCCT

Figure 5

TGTTTTAGTG TATTCTTTCG CCTCTTTCGT

TTTAGGTTGG

1251	TGCCTTCGTA	GTGGCATTAC	GTATTTTACC	CGTTTAATCG	AAACTTCCTC
1301	ATGAAAAAGT	CTTTAGTCCT	CAAAGCCTCT	GTAGOOGTTG	CTACCCTCGT
1351	TOOGATGCTG	TCTTTCGCTG	CTGAGGGTGA	OGATOCCGCA	AAAGOGGOCT
1401	TTAACTCCCT	GCAAGCCTCA	GOGACOGAAT	ATATOGGTTA	TEOGTECCCC
1451	ATGGTTGTTG	TCATTGTOGG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCACCTCG	AAAGCAAGCT	GATAAACCGA	TACAATTAAA	GGCTCCTTTT
1551	GCAGCCTTTT	TTTTTGGAGA	TTTTCAACGT	GAAAAAATTA	TTATTCGCAA
1601	TICCTITAGT	тептестте	TATTCTCACT	COCCTICAMAC	TGTTGAAAGT
1651	TGTTTAGCAA	AACCCCATAC	AGAAAATTCA	TTTACTAACG	TCTGGAAAGA
1701	CGACAAAACT	TTAGATCGTT	AOGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGOGT	TGTAGTTTGT	ACTEGTGACG	AAACTCAGTG	TTACGGTACA
1801	TGGGTTCCTA	TTGGGCTTGC	TATCCCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGG[GGCGGT	TOTGAGGGTG	GOOGTTCTGA	GEGTGGCCGT	ACTAAACCTC
1901	CTGAGTACGG	TGATACACCT	ATTCCGGGGCT	ATACTTATAT	CAACCCTCTC
1951	GACGGCACTT	ATCCCCCTCCG	TACTGAGCAA	AACCCCCTA	ATOCTAATOC
2001	TTCTCTTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
2051	GGTTCCCGAAA	TAGGCAGGGG	GCATTAACTG	TTTATACGGC	CACTGTTACT
2101	CAAGGCACTG	ACCCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TATGACGCTT	ACTOGAACOG	TAAATTCAGA	GACTGCGCTT
2201	CAAGGCACTG	ACCCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TOCCTCAACC	TOCTGTCAAT	GCTGGCGGGG	ecticitecties
2201	TOCATTOTOG	CTTTAATCAA	GATOCATTOG	TTTGTGAATA	TCAAGGCCAA
2251	TOGTCTGACC	TGCCTCAACC	TOCTGTCAAT	OCTOOCCOCC	ecticlegree
2301	TEGITCTEGT	GEOGRACICIE	AGGGTGGTGG	CTCTGAGGGT	egogetticte
2351	AGGGTGGCGG	CTCTGAGGGA	eecegiiccg	GIGGIGGCIC	театтоозат
2401	GATTTTGATT	ATGAAAAGAT	GGCAAACGCT	AATAAGGGGG	CTATGACCGA
2451	AAATGCCGAT	GAAAACGCGC	TACAGTCTGA	OGCTAAAGGC	AAACTTGATT
			Figure 5		

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2501	CTGTCGCTAC	TGATTAOGGT	GCTGCTATCG	ATGGTTTCAT	TGGTGACGTT
2551	TOOGGOOTTG	CTAATGGTAA	TOGTOCTACT	GGTGATTTTG	CTGGCTCTAA
2601	TTCCCAAATG	CCTCAAGTCG	GTGACGGTGA	TAATTCACCT	TTAATGAATA
2651	ATTTCCGTCA	ATATTTACCT	TOOCTOCCTC	AATOGGTTGA	ATGTCGCCCT
2701	TTTGTCTTTA	GOGCTGGTAA	ACCATATGAA	TTTTCTATTG	ATTGTGACAA
2751	AATAAACTTA	TICOGIGGIG	TCTTTGCGTT	TCTTTTATAT	GTTGCCACCT
2801	TTATGTATGT	ATTTTCTACG	TTTGCTAACA	TACTGCGTAA	TAAGGAGTCT
2851	TTATCATGCC	AGTTCTTTTG	GGTATTCCGT	TATTATTGCG	TTTCCTCGGT
2901	ттесттете	TAACTTTGTT	COGCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTCGGTAAG	ATAGCTATTG	CTATTTCATT	GTTTCTTGCT	CTTATTATTG
3001	GGCTTAACTC	AATTCTTGTG	GGTTATCTCT	CTGATATTAG	CGCTCAATTA
3051	COCTCTGACT	TTGTTCAGGG	TGTTCAGTTA	ATTCTCCCCGT	CTAATGCGCT
3101	TCCCTGTTTT	TATGTTATTC	TCTCTGTAAA	GECTECTATT	TTCATTTTTG
3151	ACGTTAAACA	AAAAATCGTT	TCTTATTTGG	ATTGGGATAA	ATAATATGGC
3201	TGTTTATTTT	GTAACTGGCA	AATTAGGCTC	TOGAMAGAOG	CTOGTTAGOG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	GGTGCAAAAT	AGCAACTAAT
3301	CTTGATTTAA	GGCTTCAAAA	OCTOCCGCAA	GTOGGGAGGT	TOGOTAAAAC
3351	GOCTOGOGIT	CTTAGAATAC	COGATAAGCC	TTCTATATCT	GATTTGCTTG
340	CTATTGGGGG	CGGTAATGAT	TOCTACGAATO	AAAATAAAA	COGCTTGCTT
345	1 GITCTOGATG	AGTGCGGTAC	TTGGTTTAAT	ACCOGTTCTT	GGAATGATAA
350	1 GGAAAGACAG	CCGATTATTG	ATTEGTTTCT	ACTECTOST	AAATTAGGAT
355	1 GGGATATTAT	тттсттетт	CAGGACTTAT	CTATTGTTGA	TAAACAGGCG
360	1 CGTTCTGCAT	TAGCTGAACA	TGTTGTTTAT	TGTCGTCGTC	TGGACAGAAT
365	1 TACTITACCI	TTTGTCGGTA	CTTTATATTC	TCTTATTACT	GGCTCGAAAA
370	1 тасстстасс	TAAATTACAT	спесств	TTAAATATGG	GATTCTCAA
375	1 TTAAGCCCT/	CTGTTGAGCG	TTGGCTTTAT	ACTGGTAAG/	ATTTGTATAA
380	1 OGCATATGA	r actaaacage	CTTTTTCTAG	TAATTATGAT	TCCGGTGTTT

Figure 5

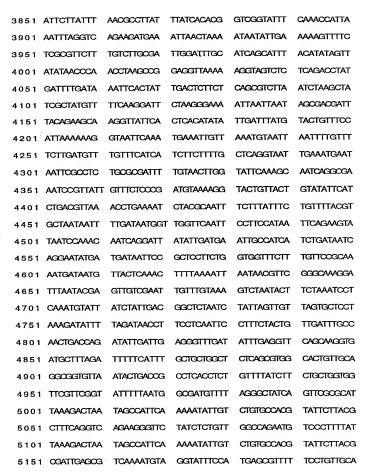


Figure 5

5201	ATGGCTGGCG -	GTAATATTGT	TCTGGATATT	ACCAGCAAGG	COGATAGTTT
5251	GAGITICTICT	ACTCAGGCAA	GTGATGTTAT	TACTAATCAA	AGAAGTATTG
5301	CTACAACGGT	TAATTTGCGT	GATGGACAGA	CTCTTTTACT	COGTTCCCCTTC
5351	ACTGATTATA	AAAACACTTC	TCAAGATTCT	GGOGTACOGT	TOCTGTCTAA
5401	AATOCCTTTA	ATCCGCCTCC	TGTTTAGCTC	COGCTCTGAT	TOCAAOGAGG
5451	AAAGCACGTT	ATACGTGCTC	GTCAAAGCAA	CCATAGTACG	OGCOCTIGITAG
5501	CGGCGCATTA	AGCGCGGCGG	GIGIGGIGGI	TACGCCCAGC	GTGACCGCTA
5551	CACTTGCCAG	CGCCCTAGCG	COCCECTOCIT	TOGOTTTOTT	COCTTOCTTT
5601	CTCGCCACGIT	TOGOCGGCTT	TOCCOCTICAA	GCTCTAAATC	GGGGGCTCCCC
5651	TTTAGGGTTC	CGATTTAGTG	CTTTACGGCA	CCTTCGACCCC	AAAAAACTTG
5701	ATTTGGGTGA	TGGTTCACGT	AGTGGGCCAT	CCCCTGATA	GACGGTTTTT
5751	OGCCCTTTGA	OGTTGGAGTC	CACGTTCTTT	AATAGTGGAC	TCTTGTTCCA
5801	AACTGGAACA	ACACTCAACC	CTATCTCGGG	CTATTCTTTT	GATTTATAAG
5851	GGATTTTGCC	GATTTOGGAA	OCACCATICAA	ACAGGATTTT	COCCUCACION
5901	GGCAAAACCAG	OGTOGACCOC	TTGCTGCAAC	TCTCTCAGGG	OCAGGCGGTG
5951	AAGGGCAATC	AGCTGTTGCC	OGTOTOGOTG	GTGAAAAGAA	AAACCACCCT
6001	GGCGCCCAAT	ACGCAAAACCG	CCTCTCCCCCG	COCCTTO	GATTCATTAA
6051	TOCAGCTOGC	ACGACAGGTT	TOOOGACTOG	AAAGOGGGCA	GTGAGCGCAA
6101	CGCAATTAAT	GTGAGTTAGC	TCACTCATTA	GGCACCCCAG	GCTTTACACT
6151	TTATGCTTCC	GECTEGTATG	TIGIGIGGAA	TIGIGAGOGG	ATAACAATTT
6201	CACACAGGAA	ACAGCTATGA	CCATGATTAC	GAATTOGAGC	TOGGTACCCG
6251	GOGATOCTCT	AGAGTOGACC	TECAGECATE	CAAGCTTGGC	ACTGGCCGTC
6301	GTTTTACAAC	GTOGTGACTG	GGAAAACCCT	GGOGTTACCC	AACTTAATOG
6351	CCTTGCAGCA	CAATCCCCTT	TOGOCAGCTG	GOGTAATAGC	GAAGAGGCCCC
6401	GCACCGATCG	COCTTCCCAA	CAGTTGCGCA	GOCTGAATGG	CGAATGGCGC
6451	THECCIGGT	TTCCGGCACC	AGAAGCGGTG	CCGGAAAGCT	GECTEGAGTG
6501	COGATICTTCCT	GAGGCCGATA	occircatocal	COCCTCAAAC	TEGCAGATEC

Figure 5

6551	ACCIGITACCIA	TGOGCOCATC	TACACCAACG	TAACCTATCC	CATTACGGTC
6601	AATCCCCCCTAA	TTGTTCCCAC	GGAGAATOOG	ACGCGTTGTT	ACTOGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GECTACAGGA	AGGCCAGACG	CGAATTATTT
6701	TTGATGGCGT	TOCTATTGGT	TAAAAAATGA	GCTGATTTAA	CAAAAATTTA
6751	ACGCGAATTT	TAACAAAATA	TTAACGTTTA	CAATTTAAAT	ATTTGCTTAT
6801	ACAATCTTCC	TGTTTTTGGG	GCTTTTCTGA	TTATCAACOG	GGGTACATAT
6851	GATTGACATG	CTAGTTTTAC	GATTACCGTT	CATCGATTCT	спаптаст
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGCCT	TTGTAGATCT	CTCAAAAATA
6951	GCTACCCTCT	COCGCCATGAA	TITATCAGCT	AGAACGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COGGCCTTTC	TCACCCTTTT	GAATCTTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	GOGTTGAAAT	AAAGGCTTCT	CCCCCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	COGATTTAGC	TTTATGCTCT	GAGGCTTTAT

Figure 5

COMPLEMENTARY TO M₁₃

POSITION 645	5 ' 3' AGCAACACTATCATA	POSITION 631	M ₁₃ /1
615	ACGACGATAAAAACC	601	M ₁₃ /2
585	TTTTGCAAAAGAAGT	571	M ₁₃ /3
555 /	AATAGTAAAATGTTT	541	M ₁₃ /4
525	CAATACTGOGGAATG	511	M ₁₃ /5
495	TGAATCCCCTCAAA	481	M ₁₃ /6
465	AGAAAAOGAGAATGA	451	M ₁₃ /7
435	CAGGTCTTTACCCTG	421	M ₁₃ /8
405	AGGANAGOGGATTIGC	391	M ₁₃ /9
375	AGGAAGOCCGAAAGA	361	M ₁₃ /10

COMPLEMENTARY TO SS PHAGE DNA

POSITION	5' * * 3'	POSITION	
351	ATATTTGAAGTCTTT	366	M ₁₃ /11
371	TCTTTTTGATGCAAT	386	M ₁₃ /12
391	CTATAATACTCAGGG	406	M ₁₃ /13
411	TGATTTATGGTCATT	426	M ₁₃ /14
431	GTTTAAAGCATTTGA	446	M ₁₃ /15
451	TATTTATGACGATTC	466	M ₁₃ /16
471	TATOCAGTOTAAACA	486	M ₁₃ /17
491	CTCTGGCAAAACTTC	506	M ₁₃ /18
511	TCGCTATTTTGGTTT	526	M ₁₃ /19
531	AAACGAGGGTTATGA	546	M _{13/2} 0

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence



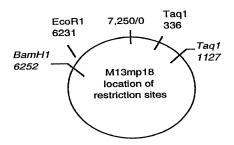


Figure 7

Appropriate M13mp18 Restriction Sites



Lane 1: from calf thymus + Taq digested mp18 amplification reaction

Lane 2: from Taq digested mp18 amplification reaction

Lane 3: from calf thymus amplification reaction

Lane 4: øX174 Hinf1 size marker

Figure 8

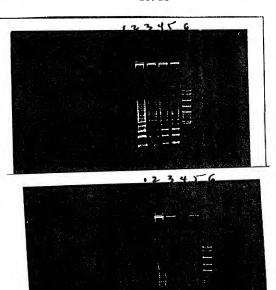


Lane 1: no template

Lane 2: mp18 template, phosphate buffer

Lane 3: Mspl/pBR322 size marker Lane 4: mp18 template, MOPS buffer

Figure 9



Top= (+) Template Bottom= (-) Template

Lane 1: phosphate buffer

Lane 2: MES Lane 3: MOPS Lane 4: DMAB Lane 5: DMG

Lane 6: pBR322/Mspl size marker

Figure 10



Lane 1: DMAB buffer, no template

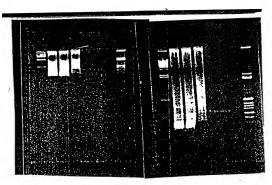
Lane 2: DMAB buffer, mp18 template Lane 3: DMG buffer, no template

Lane 4: DMG buffer, mp18 template

Lane 5: No reaction

Lane 6: 200 ng Taq I digested mp18 size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

Lane 1: lambda Hind III marker

Lane 2: Amp/Untreated

Lane 3: Amp/Kinased

Lane 4: Amp/Kinased/Ligated

Lane 5: PCR/Untreated

Lane 6: PCR/Kinased

Lane 7: PCR/Kinased/Ligated

Lane 8: øX174/Hinf1 marker

Figure 12

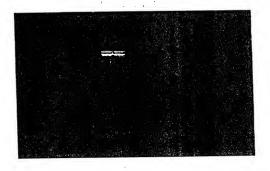
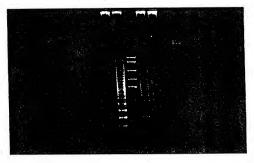


Figure 13

1 2 3 4 5 6



Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

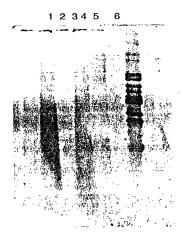
Lane 4: Primers + RNA

Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 14



Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA

Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 15

Figure 16

580

600 62 Wavelength (nm)

620

640

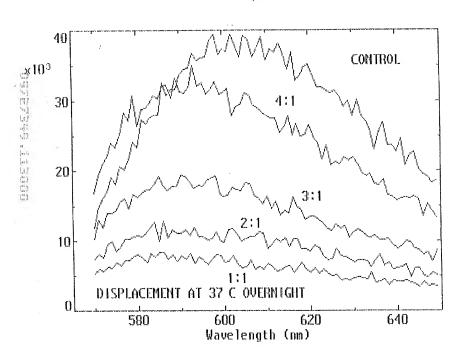


Figure 17

pIBI 31-BH5-2

fmet AUG of Lac z $$\{\mbox{T7 Promotor region}....$ LAC PROMOTOR..ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA

oligo 50-mer 3'- tac t*aa t*gc ggt* ct*a t*ag t*Vt aat* tat* gct* gag t*ga t*at* c-5' 10 base insert

T7 RNA Start { «« T3 Promotor Region } IGGG CTC ICCT TTA GTG ACG GTT AAT ...»» } «- T3 Start Signal

pIBI 31 BSII/HCV

{<- T7 Promotor Region }
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....

- T7 Start Signal

5'-ct'a t'ag t'ga gt'c gt'a tt'a at'..........

Figure 18